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DEVICE FOR CULTIVATING CELLS IN A MONOLAYER
[Ustanovka dlya kul'tivirovaniya kletok v monosloye]

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TITLE (54): DEVICE FOR CULTIVATING CELLS IN A MONOLAYER

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SPECIFICATIONS

The invention relates to cell biology and, in particular, to equipment for growing cells of animals and humans and it may be used in medicine, virology, and veterinary medicine.

Most similar in technical essence to the proposed invention is a device for growing cells in a monolayer, containing a culture vessel with packing for holding cells and piping for feeding and discharging the culture medium [1].

The known system also contains a temperature-control circuit and an external circulation circuit, including a pH meter, a peristaltic pump, and a three-way valve.

The cells, attached to an immobile packing material, are constantly washed with fresh culture medium. The peristaltic pump feeds in culture fluid, depending on the pH of the nutrient medium.

The shortcoming of the known system is that, in the circulation of the nutrient medium, cells that are not attached to the packing are destroyed by the operation of the peristaltic pump. Another problem with this device is the small interphase contact surface of the gas with the culture fluid, which makes intense gas exchange in the system difficult.

The object of this invention is to increase the growing efficiency by increasing the interphase contact surface of the gas with the culture fluid and reducing traumatism to the cells.

This object is achieved in that the proposed device is equipped with an additional vessel with packing which communicates with the lower portion of the main culture vessel, a system for feeding and discharging gas in the vessels for contact between it and the culture fluid and moving it from one vessel to the other, and a device for regulating the feeding and discharge of the gas in the vessels.

The regulator device has gages for the level of culture fluid in the vessels, a switching unit, a four-way electropneumatic valve with an actuating mechanism that is connected to the vessels. A level gage is connected to the input of the switching unit and the output of this unit is connected to the actuating mechanism of the four-way electropneumatic valve.

Figure 1 shows a diagram of the device described here.

The device contains two culture vessels **1** with glass packing **2** for holding the cells. At their bottoms, vessels **1** are connected by line **3**. Culture fluid is fed in through line **4** and it is discharged through line **5**.

The device also contains four-way electropneumatic valve **6** with its actuating mechanism, switching unit **7**, and gage **8** for the level of the culture fluid in the vessels.

Switching unit **7** consists of a relay circuit **9** and a switching circuit **10**.

Four-way electropneumatic valve **6**, switching unit **7**, and culture fluid level gage **8** form a device for regulating the feed and discharge of gas in the vessels.

The device is equipped with a system for feeding and discharging gas into and from the vessels for contact between the gas and the culture fluid in the vessels and for moving the fluid from one vessel to the other. This system includes gas feed line **11** and discharge line **12** attached to the top portion of vessels **1**, which are connected to pressure line **13** for delivering the gas mixture, and discharge line **14**.

Pressure line **13** is connected to microcompressor **15**. A reservoir **16** with inoculum and nutrient is connected to one of the culture vessels **1**.

A thermal jacket **17** is used to control the temperature of the vessels. Cooling elements **18** are installed in lines **12** for condensing culture fluid vapor that enters the lines along with the discharge gas

and returning the condensed fluid to vessel **1**.

The device operates as follows.

Vessels **1**, 3/4 filled with packing, is filled with nutrient and inoculum up to level indicator **8**. The cells are held there for an amount of time required to attach them to packing **2**. Then the medium is removed from both vessels to 1/2 their volume. After the cells are attached (the attachment time is determined in advance by any known method, such as using a Petri dish), the gas mixture is fed under excess pressure into one of the culture vessels **1**. Under the gas pressure, the column of fluid drops in one of the vessels **1** and it rises in the other to a level determined by gage **8**. When the fluid leaves vessel **1**, a film of culture fluid remains on packing **2**, covering the cells and providing intense mass exchange.

The fluid level rises in the second vessel **1** to the level of gage **8**, which is actuated and sends a signal to switching unit **7**, which actuates four-way electropneumatic valve **6** by means of its actuating mechanism.

After valve **6** is actuated, the fluid drops in second vessel **1** and rises in first vessel **1** to the level of gage **8**, which is actuated once again and the cycle repeats.

Example. Two 500-ml culture vessels are filled with packing in the form of Raschig rings measuring 5x4x6 mm made of C-52 glass. The vessels are then sterilized and filled with medium at a 1:1 ratio. Needle + 199 with 10% normal bovine serum.

Liver cells from a Chinese hamster were placed in the medium at a concentration of 90-100 thousand cells per 1 ml medium. A mixture of air and 5% CO_2 is initially fed into the entire volume of the apparatus by the microprocessor to stabilize the pH at 7.2-7.4.

Under these conditions, the inoculate is held for the 4-5 hours required for cell attachment, after which half the volume of the entire medium is poured in and the pumping system is attached. The transfer cycle (from one vessel to the other) takes 8-10 minutes.

The complete cell-growth cycle to form a monolayer is completed in 72 hours, after which the cells are harvested, the harvest being 1-1.5 million cells per 1 ml medium.

The proposed invention increases the biomass yield by creating "soft" hydrodynamic conditions for the growth of cells that are attached to an immobile packing, by increasing the actively utilized substrate surface, by increasing the size of the device in the form of a second culture vessel, by improving the mass-transfer conditions, and by eliminating the need to use a special circulation circuit with

a peristaltic pump.

Claims

1. A device for cultivating cells in a monolayer containing a culture vessel with a packing for attaching the cells and piping for feeding and discharging the culture fluid, characterized in that, in order to increase efficiency in cultivating cells by increasing the interphase contact surface of the gas with the culture fluid and to reduce cell traumatism, the device is equipped with an additional vessel with packing that is connected to the lower portion of the main culture vessel, a system for feeding and discharging the gas in the vessels for contact of the gas with the culture fluid and for moving it from one vessel to the other, and a device for regulating the feed and discharge of the gas to and from the vessels.

2. A device as recited in Claim 1, characterized in that the regulator device includes gages for the level of culture fluid in the vessels, a switching unit, and a four-way electropneumatic valve with an actuating mechanism connected to the vessels, whereby the level gages are connected to the input of the switching unit and the output of the switching unit is connected to the actuating mechanism of the four-way electropneumatic valve.

Sources of information for use by examiners

1. Wöhler, W. Culturing of Normal Dipliod Cells on Glass Beads Using a Novel Type of Culture Vessel. Exp. Cell Res., 74, 571, 1972.